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THE EFFECT OF EXPOSURE TO LIGHT DURING THE DARK PHASE OF THE CIRCADIAN CYCLE ON IOP AND AQUEOUS FLOW IN NEW ZEALAND WHITE RABBITS

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1993

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A Thesis Submitted to the
Yale University School of Medicine
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Degree of Doctor of Medicine

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ABSTRACT

THE EFFECT OF EXPOSURE TO LIGHT DURING THE DARK PHASE OF THE CIRCADIAN CYCLE ON IOP AND AQUEOUS FLOW IN NEW ZEALAND WHITE RABBITS

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Rabbits have circadian rhythms of IOP and aqueous flow; both IOP and flow are lowest early in the light phase and highest early in the dark phase. The phase of the light:dark cycle sets the phase of both rhythms, and both rhythms persist in constant dark. Many circadian rhythms are abolished by constant light, and Rowland, et al. (28), showed that exposing rabbits to constant light for 4 days abolished the circadian rhythm of IOP. Light also has acute effects on some circadian rhythms; the dark phase increase of the circadian rhythm of melatonin production and its biosynthetic enzyme, N-acetyltransferase, in the pineal and in the retina is rapidly decreased by exposure to light during the dark phase of the circadian cycle.

In order to determine the acute effect of light during the dark phase of the circadian cycle on IOP, IOP was recorded in rabbits previously entrained to 12L:12D after lights were turned on during the dark. This reduced IOP by an average of 5.5 mmHg. When the lights were turned off 4 hours later, IOP rebounded rapidly to control dark phase pressure. Superior cervical ganglionectomy or preganglionic section of the cervical sympathetic trunk (decentralization) markedly reduced the response of IOP to light during the dark phase. Aqueous flow, measured by the intravitreal depot method, did not change when rabbits were exposed to light during the dark phase.

Light has two effects on IOP in rabbits: (1) it sets the phase of the circadian rhythm of IOP; (2) it rapidly and reversibly decreases IOP during the dark phase of the circadian cycle.

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INTRODUCTION

Variations in physiological processes with respect to time have long been recognized in animals and plants. The earth, spinning on its axis approximately once every 24 hours, submits plants and animals to highly predictable daily rhythms of light and temperature. The availability of food and the activity of predators are in turn affected by these periodic variations. It is not surprising, therefore, to discover that the behavior and metabolism of organisms follow a 24-hour schedule. That this schedule is not the result of a passive response of the organism is evident by the observation that, when an organism is isolated from all environmental time cues--when light, food, temperature, and sound are kept constant around the clock--the majority of its rhythms persist. This "free-running" period is usually close to but not exactly 24 hours (1).

The physiological system responsible for measuring time and synchronizing an organism's internal processes with the daily events in its environment is known as the circadian timing system. The word ***circadian*** (Latin: *circa*=about; *dies*=day) was coined by Franz Halberg in 1959 to describe the approximately 24-hour cycles that are endogenously generated by an organism (2). Twenty-four-hour rhythms in the activities of plants and animals were observed many centuries ago; written records of Alexander the Great from the fourth century B.C. documented the daily movements of leaves and flower petals. Androsthenes reported that the tamarind tree, known at that time primarily for its laxative fruit, opened its leaves during the day and closed them at night (3). However, the endogenous origin of

these circadian rhythms was not recognized until 1792 when Jean Jacques d'Ortous de Mairan performed an experiment and observed that a heliotrope plant, which opens its leaves and pedicels during the day and folds them at night, continued to open its leaves during the day and fold them for the entire night when transferred to a place where sunlight could not reach it. This was the first demonstration that circadian rhythms persist in the absence of environmental light. De Mairan also proposed at that time that another experiment be performed--reverse light and dark and examine the response of the plant--to determine whether the phase of light:dark cycle acts as a time cue for the plant to open and close its pedicels. His ideas, however, were to remained unnoticed for the next few decades. In 1832, Augustin de Candolle demonstrated that circadian clocks would "free-run" with their own endogenous period when they were no longer synchronized to a 24-hour light-dark cycle (4). By studying bees, von Frisch and Beling concluded in 1929 that organisms could truly measure the passage of time (5).

The endogenous nature of circadian rhythms in mammals was first recognized in 1866 when William Ogle (6) made careful observations of the daily body temperature rhythm in man and concluded that "There is a rise in the early mornings while we are still asleep, and a fall in the evening while we are still awake, which cannot be explained by reference to any of the hitherto mentioned influences. They are not due to variations in light; they are probably produced by periodic variations in the activity of the organic functions." This endogenous property of mammlian circadian clocks was later extensively studied by Curt Richter, who in 1922

demonstrated it in the rat and showed that they were synchronized by light-dark cycles and the time of feeding (7). The endogenous nature of the circadian system in humans was confirmed only more recently. In 1962, Aschoff and Wever (8) isolated individuals in a sealed cellar below a Munich hospital for 8-19 days in the absence of any environmental time cues, and Siffre (9) lived alone in an underground cavern for 2 months. Both experiments revealed that the spontaneous or free-running period (i.e. the endogenous period when they were no longer synchronized to a 24-hour light-dark cycle) of the human activity-rest cycle was greater than 24 hours, averaging 25 hours, indicating that humans, like other animals, have an endogenous circadian system. Subsequently, various investigators have documented circadian rhythms in hundreds, if not thousands, of biological variables such as the sleep-wake cycle, feeding and drinking, thermoregulation, endocrine rhythms, renal function, and reproduction. Conroy and Mills (10), in Human Circadian Rhythms, documented many of such rhythms in humans. As the documentation of rhythms in various human physiological systems proceeded, it became apparent that it was often more significant to find no circadian rhythm in a physiological variable than to find one.

As long ago as 1918 neuropathological studies in humans had indicated that an area in the anterior hypothalamus was important in the regulation of sleep (11). Nauta (12) in 1946 further localized this "sleep center" to the suprachiasmatic-preoptic area on the basis of lesioning studies in rodents. Richter suggested in his study that the only lesions that would disrupt circadian rhythmicity in wheel-running behavior in rodents were those placed in the anterior-

ventral hypothalamus (13, 14). In 1972, two groups of researchers independently identified a small, bilateral pair of nuclei in the anterior hypothalamus, the *suprachiasmatic nuclei* (SCN), as a potential circadian pacemaker (15, 16).

Since circadian clocks, which free-run under constant conditions, are entrained to a 24-hour period in natural environments, it is clear that circadian systems are sensitive to some environmental time cue. Studies of animals in rigorous isolation have made it apparent that only certain specific environmental variables are capable of acting as circadian time cues. In 1951, Aschoff coined the term ***zeitgeber***, from the German, meaning "time-giver," to describe such periodic environmental cycles (17, 18). Although *zeitgeber* is the most frequently used term, a number of others appear in the literature, including "synchronizer," "entraining agent," and "time cue." To demonstrate that an oscillation in an environmental variable acts as a time cue to the circadian system, one must show that the following criteria are met (1):

(1) *Absence of other time cues.* The monitored circadian rhythm must be free-running with an independent period before the time cue is imposed upon the animal and must resume free-running after the time cue is removed.

(2) *Period control.* Once the animal is exposed to the environmental cycle, the period of the circadian rhythm must adjust so as to equal the period of the environmental cycle.

(3) *Stable phase relationship.* A stable and reproducible relationship must emerge and be maintained between the timing of the observed rhythm and the timing of the zeitgeber; the rhythm's occurrence must thus be independent of clock time and dependent only on the

imposed time cue.

(4) *Phase control.* When the time cue is removed, the rhythm must start to free-run from a phase determined by the environmental cycle and not by the rhythm prior to entrainment.

CIRCADIAN RHYTHM OF IOP

It has long been known that intraocular pressure (IOP) does not remain permanently at a constant level, but displays instead certain characteristic daily fluctuations. In humans, IOP shows daily rhythmicity that can be especially marked in pressure pathologies (19-24). Often there is an early morning rise in pressure, which is critical in glaucoma, followed by lowest pressures in the evening, during onset of normal sleep. Healthy human eyes are thought to have daily ranges of 3 to 5 mmHg whereas subjects with consistent ranges of 10+ mmHg are generally suspected to be pathological. Ericson found in 1958 that, in humans, IOP reaches a high value between 8 a.m. and 12 p.m. noon, and thereafter drops throughout the day to reach a minimum at 12 a.m. midnight, with a decrease of 19% in IOP. IOP begins to rise again beginning as early as 4 a.m. (19).

In 1975 Katz et al. reported that New Zealand White rabbits, housed in L:D schedule of 0700 to 1730 light:1730 to 0700 dark-when transfered to a room with L:D schedule of 0730 to 2330 light:2330 to 0730 dark for IOP measurement-exhibited "a circadian rhythm of IOP characterized by a low pressure at night and rising to the highest during the day." (25) In 1977, Vareilles et al. developed

a laboratory method using a pneumatic tonometer to systematically measure the normal IOP in the unanesthetized rabbit in constant light, and found it to lie between 15 and 23 mmHg (26). Bar-Ilan later performed an experiment studying rabbit IOP in a 12-month period and showed the annual curve to contain two peaks of IOP, during the summer and winter seasons (27).

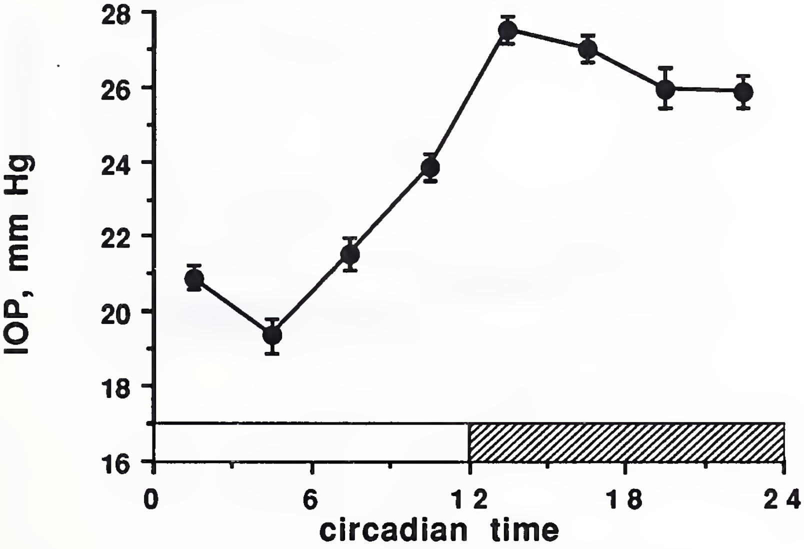

Fig. 1: Circadian rhythm of IOP in rabbits.

The development of an animal model of circadian rhythm of IOP may facilitate investigations of the daily IOP oscillation in humans. Over the years, the rabbit has been the animal used frequently in glaucoma research. Rowland, Potter, and Reiter (28), using carefully maintained environmental lighting conditions, first developed a circadian model of IOP in this animal. In rabbits entrained to a 12-hour light:12-hour dark cycle, they found a highly predictable maximum of 27 mm Hg and minimum of 15-16 mmHg in IOP which closely correlated with the onset of darkness and light,

respectively. The daily spontaneous variations in IOP, once entrained, were maintained in constant darkness, but were abolished by constant light, as is similarly seen in many other physiologic processes of the mammalian systems.

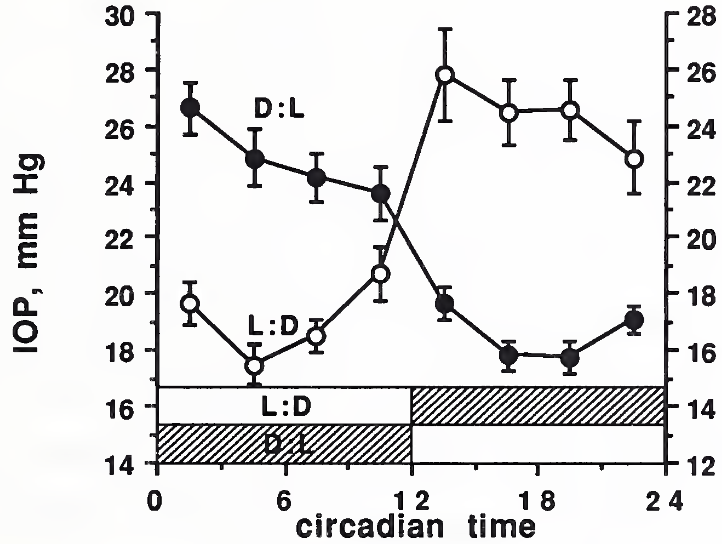

Fig. 2: Circadian rhythm of IOP with respect to the light:dark cycle.

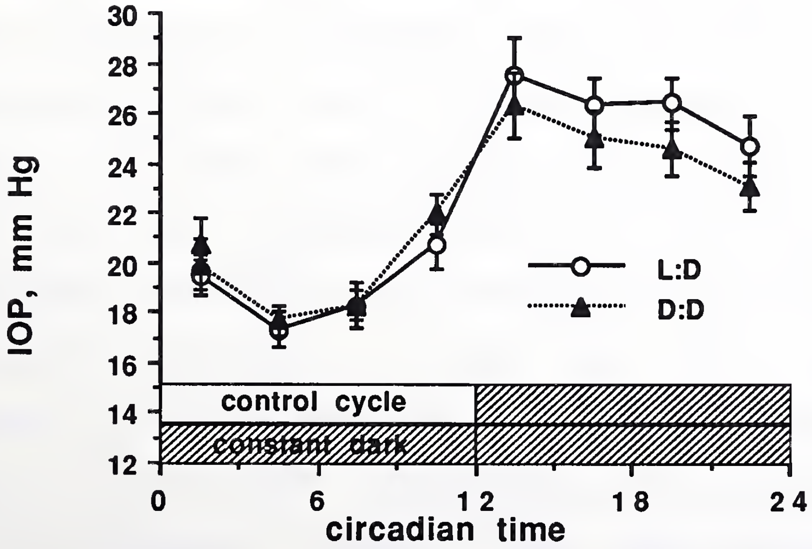

Fig. 3: IOP light:dark vs. dark:dark (n=11).

Gregory et al. (29) later confirmed this finding (Figures 1) and in addition found that superior cervical ganglionectomy depressed the dark-phase increase in IOP but had little effect on IOP during the light phase (Figure 4). They further showed that the phase of IOP is determined by the phase of the light:dark cycle, and the rhythm of IOP persisted in constant dark (Figures 2-3); thereby establishing the cycle of light and dark as the *zeitgeber* for entrainment of the rhythm of IOP, and the rhythm of IOP as circadian (29). Braslow et al. showed that preganglionic section of the cervical sympathetic trunk ("decentralization") markedly reduced the dark-phase increase in IOP (Figure 5) while having little effect on IOP during the light phase (30). Yoshitomi et al. (31), and Liu et al. (32), demonstrated that aqueous norepinephrine and cAMP were higher during the dark in similarly entrained animals, and Kiuchi et al. found that these rhythms persisted in constant dark (33). In addition, superior cervical ganglionectomy (CGX) and decentralization (DX) reduced aqueous norepinephrine concentration during both light and dark, while CGX, DX, and pretreatment with 0.1% timolol (a beta-adrenergic antagonist) reduced the dark phase increase of aqueous cAMP. Gregory found that timolol reduced IOP in rabbits during the dark phase only (34). Recently, Kiuchi et al. used alpha$_2$-adrenergic antagonists, yohimbine and rauwolscine, and the alpha$_1$-adrenergic antagonist, bunazosin, to explore the role of alpha-adrenergic receptors in the regulation of the circadian rhythms of IOP and aqueous flow in rabbits. Their results were inconclusive for the role of alpha$_2$-adrenergic receptors, while alpha$_1$-adrenergic receptors increased IOP by a mechanism that has yet to be identified (35). The

studies above suggest that beta-adrenergic receptors mediate the increase in IOP during the dark through the release of adrenergic agonists and that alpha_1-adrenergic receptors increase IOP by an unknown mechanism. The role of alpha_2-adrenergic receptors has yet to be clarified.

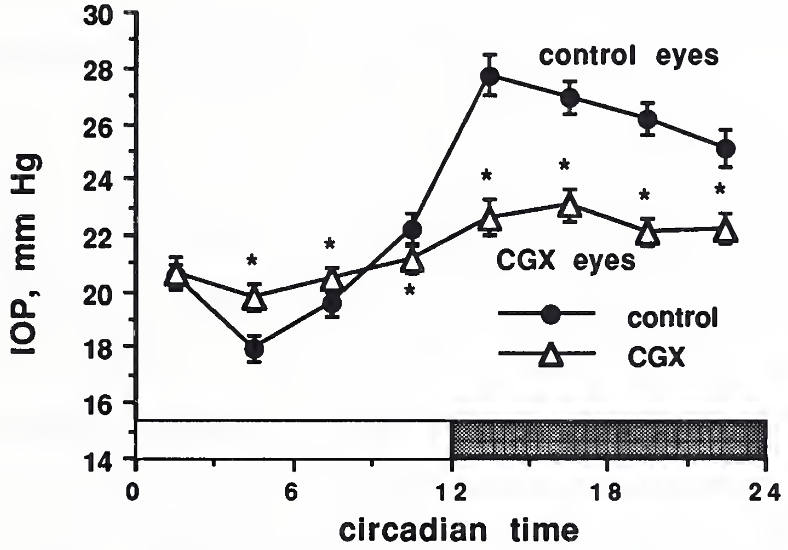

Fig. 4: The effect of unilateral superior cervical ganglionectomy on IOP (n=25).

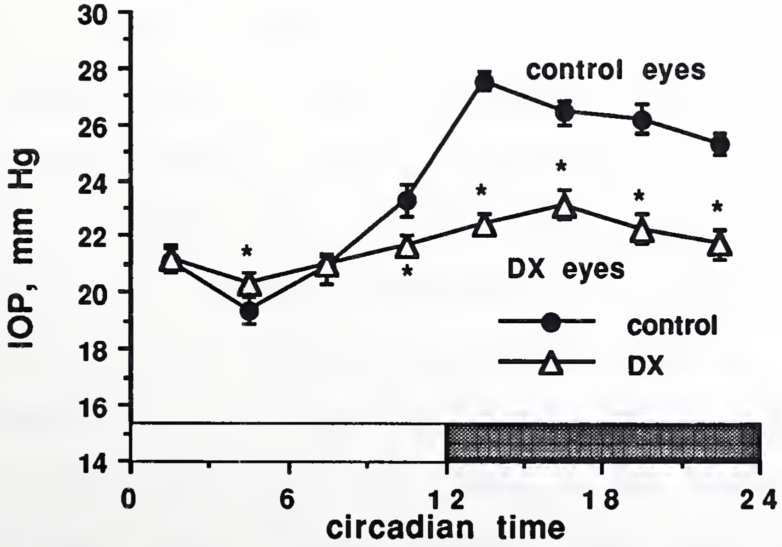

Fig. 5: The effect of unilateral decentralization of superior cervical ganglion on IOP (n=25).

All these results indicate that there is a 24 hour circadian rhythm of IOP in rabbits entrained to a 12L:12D, and that intact sympathetic innervation to the eye is required for maintenance of this rhythm of IOP.

CIRCADIAN RHYTHM OF AQUEOUS FLOW

The normal 24 hour variation in intraocular pressure can be caused by the changes in the rate of the inflow of aqueous humor, the resistance to the outflow of the aqueous humor, or episcleral venous pressure. Using the suction-cup method of flow measurement described by Rosengren (36, 37), Ericson conducted studies on the 24 hour variations in the inflow of the aqueous humor in 50 nurses and found that there was a diurnal variation of inflow of aqueous which was constant during the day (11-12.5 mm^3/15 min. or 0.73-0.83 ul/min.) and dropped considerably to 1/4 the value (3-4 mm^3/15 min. or 0.2-0.27 ul/min.) at night (19). Anjou and Krakau found a strong correlation between the aqueous flare density and aqueous protein concentration in rabbits during the summer, with high values of flare and protein concentration in the morning and low values at night (38, 39). Relying on the results of Ericson and Anjou, Krakau concluded that there is a diurnal variation of aqueous flow, and that an inverse relationship exists between aqueous flow and flare (40). Reiss et al. found that in humans, aqueous flow was 45% lower during sleep than during the morning hours, with the mean flow rates of 3.1 ul/min during the morning

and 1.6 ul/min during sleep. In addition, subjects that underwent sleep-deprivation also exhibited similar diurnal variation with flow values of 3.1 ul/min in the morning and 2.3 ul/min at night during sleep deprivation. However, no significant differences of intraocular pressure at the different times of the day were observed (41). Topper and Brubaker found that timolol, a nonselective beta-adrenergic blocker, reduced the daytime aqueous flow rate in humans by 30% while having no effect on flow at night. Epinephrine, a mixed alpha, beta-adrenergic agonist, was found to increase the flow rate by 15% during the day and 47% at night, while having no effect on IOP. Acetazolamide, a carbonic anhydrase inhibitor, reduced daytime flow rate by 20% while reducing night time flow rate only by 2%; IOP values again were not affected. The authors proposed that the rate of aqueous humor formation is stimulated by circulating endogenous epinephrine and the effect of this hormone is blocked by beta adrenergic blockers (42). Larson and Brubaker later confirmed the above findings of diurnal variation of aqueous flow and in addition found that isoproterenol, a beta-adrenergic agonist, increased flow in the normal human eye by 34% and in partially adrenergically denervated human eye (Horner's syndrome) by 50%. This finding was consistent with the hypothesis that spontaneous beta-adrenergic activity is much lower in the eye during sleep. They also concluded that, "since the diurnal rhythm of flow was present even in eyes with postganglionic Horner's syndrome, a full complement of adrenergic innervation to the eye is not required for this rhythm to occur." (43) Gharagozloo et al. showed more specifically that terbutaline sulfate, a selective beta_2-agonist,

stimulated aqueous flow in humans by 15% during sleep while having no effect on IOP or daytime flow, and attributed the effect of terbutaline on the eye to a stimulation of beta$_2$-receptors in the ciliary body (44).

Rowland et al. conducted studies of aqueous flow in New Zealand White rabbits entrained to 12L:12D cycle and determined (although he only made 2 points of measurement) that there was a light-time (1 hour before lights off) value of 1.63 ul/min and a dark-time (3 hours after lights off) value of 2.59 ul/min, and that this nearly 60% increase in flow in the dark correlated well with parallel increases of 85% in IOP and 80% in aqueous cAMP (45). Smith et al. demonstrated that, in similarly entrained rabbits, the phase of this daily rhythm of aqueous flow (see Figure 6) is determined by the phase of light:dark cycle (Figure 7) and the rhythm persisted in constant dark. Therefore, in New Zealand White rabbits the rhythm of aqueous flow, like the rhythm of IOP, is circadian, and this rhythm of flow underlies the circadian rhythm of IOP (46).

Yoshitomi and Gregory found that during the dark the circadian rhythm of aqueous flow is decreased by topical timolol, decentralization of the superior cervical ganglion (DX), or ganglionectomy (CGX), while during the light phase CGX increased flow and timolol or DX had no effect (47). Their results suggest that part of the increase of aqueous flow during the dark is produced by adrenergic input to the ciliary processes and mediated, in part, by beta$_2$-adrenergic receptors.

Bausher et al. (48) studied the effect of alpha$_2$-adrenergic receptors on beta$_2$-adrenergic receptor-mediated stimulation of

cAMP production by intact, excised rabbit ciliary processes in vitro. They found that decreased stimulation of cAMP at high concentration of isoproterenol was blocked by the specific alpha$_2$-adrenergic antagonist, yohimbine, but not by alpha$_1$-adrenergic antagonist prazosin. Their results suggest an interaction between alpha$_2$- and beta$_2$-adrenergic receptors such that alpha$_2$-adrenergic receptor may inhibit the stimulatory effect of beta$_2$-adrenergic receptors on cAMP production by ciliary processes and therefore may play an inhibitory role on the regulation of aqueous flow.

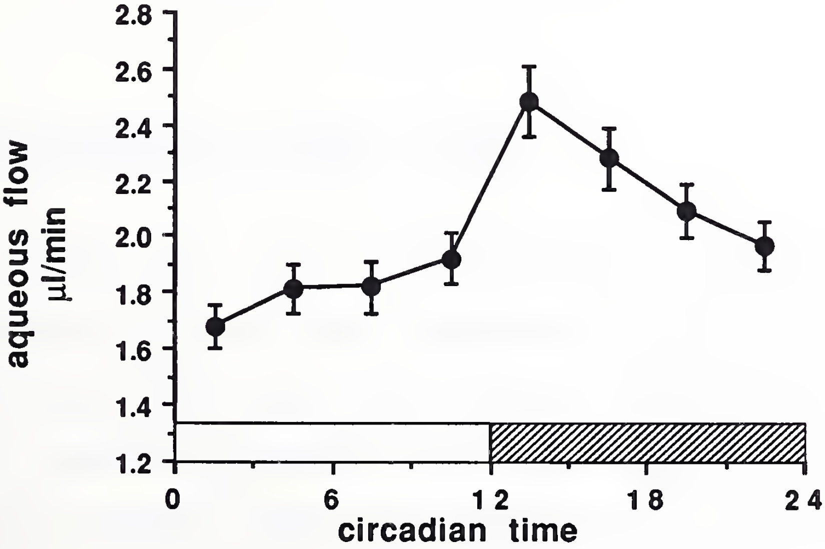

Fig. 6: Circadian rhythm of aqueous flow in rabbits.

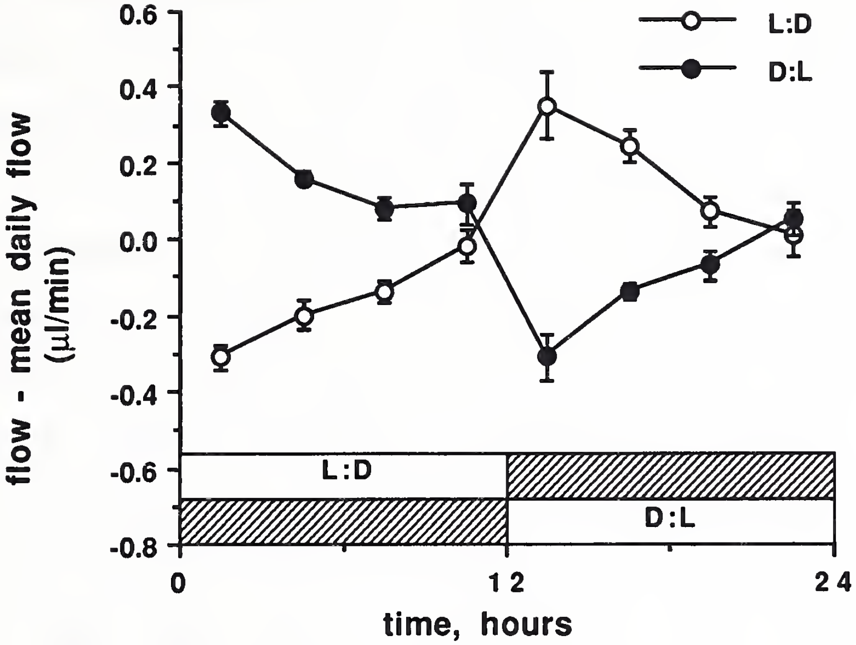

Fig. 7: Circadian rhythm of aqueous flow with respect to the light:dark cycle.

BIOSYNTHESIS OF MELATONIN

The pineal body of homiothermic vertebrates is thought to modulate at least some reproductive processes controlled by the photoperiod (49) and certain aspects of physiological rhythms (50). The pineal is implicated in the phase-timing of daily and long-term cyclic processes that are responsive to changes in the natural photoperiod and thus plays a role in maintaining the animal in harmony with its environment. In a series of experiments (51, 52), it was shown that information about environmental lighting reaches the rat pineal as follows: retina-->inferior accessory optic tract--> medial forebrain bundle-->medial terminal nucleus of the accessory

optic system-->preganglionic sympathetic tract in the spinal cord--> superior cervical ganglia-->postganglionic sympathetic fibers--> parenchymal cells of the pineal.

The biosynthesis of all pineal indoles is initiated by the uptake of circulating tryptophan into the parenchymal cells against a concentration gradient . The amino acid is then utilized for the synthesis of pineal proteins (53). That part which is destined to become serotonin or one of its derivatives is initially hydroxylated at the 5-position through the action of tryptophan hydroxylase. In the rat, the activity of this enzyme per unit weight of tissue is higher in the pineal than in any other organ (54). The resulting 5-hydroxytryptophan is rapidly decarboxylated by aromatic l-amino acid decarboxylase to form the amine 5-hydroxytryptamine or serotonin (55). In the pineal gland, serotonin is acetylated by N-acetyltransferase to form N-acetylserotonin (56, 57); this compound is then O-methylated through the action of hydroxyindole-O-methyltransferase (HIOMT) to form melatonin (56, 58).

Melatonin (N-acetyl-5-methoxytryptamine) is produced mainly by the pineal gland of mammals and amphibians. In mammals, it exerts inhibitory effects on gonads, and in fish and amphibians, it causes changes in skin pigmentation (59). Daily rhythms in the pineal content of melatonin and the biosynthetic activity supporting its synthesis have been found to be responsive to changes in environmental lighting (49). This rhythm has been found to be high during the dark and low during the light; while it persists in blinded and normal rats kept in continuous darkness, it is abolished in constant light (60).

Serotonin content (61), melatonin content (62), and activities of melatonin-synthesizing enzymes, hydroxyindole-O-methyltransferase (63), and serotonin N-acetyltransferase (64) in the rat pineal organ were shown to exhibit circadian changes mediated by sympathetic nerves whose cell bodies lie in the superior cervical gangalion.

CIRCADIAN RHYTHM OF N-ACETYLTRANSFERASE

The daily rhythms of serotonin and melatonin have been postulated to be driven by changes in N-acetyltransferase (NAT) activity (61, 62, 64, 65, 66).

In their experiment with male Osborne-Mendel rats, Klein and Weller reported a rapid increase in the activity of N-acetyltransferase in the pineal gland to values which were more than 15 times greater than the day values (64) during the first 3 hours of the dark period, whereas other researchers had shown as high as 50-fold increase during the first 4 hours in the dark (67). This daily increase and decrease of N-acetyltransferase activity, as shown by the experiment of Deguchi and Axelrod (67), is regulated by norepinephrine and mediated by cyclic AMP (68) via the beta-adrenergic receptor. The exposure of rats to continuous lighting suppressed the N-acetyltransferase rhythm. The suppression of this circadian rhythm by light seems to be mediated by an ocular route, because the removal of the eyes before light exposure permited the rhythm to persist. Ganglionectomy or decentralization of the superior cervical ganglion completely blocked the night time increase

in N-acetyltransferase activity (67, 69). This rhythm of N-acetyltransferase has the same phasing as that of melatonin (66).

Binkley et al. (70, 71) performed experiments using chicken eyes and pineal glands in parallel and found that in a light-dark cycle, dark-time NAT was 7.0-11.8 times as high as light-time NAT in the pineal, and that dark-time NAT was 2.7-2.9 times light-time NAT in the eye. Similarly, when placed in constant dark, the subjective dark-time NAT in both the pineal (70) and in the eye (72) were higher than the subjective light-time NAT, i.e. the rhythms persisted in constant dark. These experiments thus indicated that the eye also contains N-acetyltransferase whose daily activity shows a rhythmic change with respect to a daily light-dark cycle. The ocular NAT activity was subsequently localized to 41-56% in the neural retina and 42-57% in the pigmented layer.

A comparison of the characteristics of the circadian rhythms in pineal serotonin (61, 65) and N-acetyltransferase indicated that a reciprocal relation (i.e. 180 degrees out of phase) persists under normal conditions. Like the N-acetyltransferase rhythm, the most dramatic change in serotonin concentrations, a sharp decrease, occurs immediately after the lights are turned off (61). The serotonin rhythm is also suppressed by constant light, which results in continuously high values, and a rhythm in pineal serotonin persists in constant darkness and in blinded animals (65). The circadian rhythms of serotonin and N-acetyltransferase under normal conditions were explained by the following hypothesis by Klein and Weller (64). The initial event that synchronizes the rhythm, the "zeitgeber," is a dark-induced release of norepinephrine from nerve

endings. Norepinephrine stimulates adenyl cyclase in the pinealocytes (73), resulting in the increased production of adenosine 3',5'-monophosphate which stimulates N-acetyltransferase (74). Any or all of these preliminary steps could be inhibited by light acting through an ocular photoreceptor mechanism. A rapid increase in N-acetyltransferase at night produces a rapid decrease in the concentration of serotonin and results in the increased concentration of N-acetylserotonin and melatonin (74). Pineal serotonin probably decreases because serotonin production cannot increase sufficiently to keep pace with the increased rate of acetylation. This seems possible since the activity of tryptophan hydroxylase, which is apparently the rate-limiting enzyme in serotonin synthesis in the pineal gland (54, 65), is normally about as low as daytime N-acetyltransferase activity, and a diurnal rhythm in tryptophan hydroxylase has not been reported.

A circadian rhythm in norepinephrine storage in the pineal has been reported. The highest concentration of norepinephrine occurs at the end of the dark period (75). Brownstein and Axelrod found this rhythm to persist in blinded rats but was suppressed in normal rats by light (76).

DAILY RHYTHM OF HIOMT

The enzyme hydroxy-O-methyltransferase (HIOMT), like N-acetyltransferase, has been found in mammals in both the pineal gland and the retina (58, 70, 72, 77-81). In their experiments on Sprague-Dawley female rats entrained to 12L:12D (lights on @ 07:00

and off @ 19:00), Axelrod et al. found that there was a naturally occurring daily rhythm in HIOMT activity in the pineal gland with lowest activity at 12:00 and highest at 00:00 (63); in rats kept in either constant light or dark, rats kept in the dark had more than 4 (range: 2-10) times as much HIOMT activity as rats kept in light. In blinded rats, continuous light or darkness had no effect on HIOMT activity, which suggests that the retina is necessary for transmission of light messages to the pineal. Bilateral lesions of the medial forebrain bundle, which contains noradrenergic and serotonergic nerves, also abolishes the effects of environment lighting on pineal HIOMT activity (82).

THE EFFECT OF LIGHT ON PINEAL SEROTONIN DURING THE DARK PHASE OF THE CIRCADIAN CYCLE

Various studies have shown that the diurnal rhythm of pineal serotonin (63, 65, 75, 83, 84) can be modified by environmental lighting: the pineal serotonin content of rats housed under continuous illumination was the same during day and night (65). The nocturnal decline, which occurs under normal (12L:12D) lighting conditions, was prevented for 4 hours if light was extended an additional 4 hours (83, 85). Light turned on earlier than usual in the morning caused an increase in serotonin content before the expected time (83). In Illnerova's study on the dependence of pineal serotonin content on change in environmental lighting (86), 30-day-old rats were transferred from darkness to light at 11 p.m. (lights on @ 7 a.m. and off @ 7 p.m.), and from light to darkness at 1 p.m., and

killed after 13, 24, and 40 minutes in light or in darkness. The concentration of serotonin increased within 13 minutes in light (in the D-->L group) to a value similar to the content at 1 p.m. and did not further change significantly. In contrast, in darkness (in L-->D group) the decrease in pineal serotonin was significant only after 40 minutes. In the D-->L group, the change in serotonin in the 13 minutes was most dramatic during the last 4 minutes. To prove that this increase in serotonin was not due to the abrupt light during dark as a stress factor per se, another group of animals were placed in the dark and subjected to a different stress (cold temperature of 3 C), and the result showed that coldness as a form of stress did not cause an increase in pineal serotonin. Therefore, light caused a very rapid response (increase) in pineal serotonin content. Since the activity of 5-hydroxytryptophan decarboxylase (the enzyme forming serotonin) is the same in the pineal during day and night, and doubles only in rats kept for a long time in light (87), it is not probable that the increase in serotonin is due to a higher rate of synthesis; rather, it may be more likely due to a decreased loss from the pineal (e.g. decreased conversion to other products such as melatonin).

THE EFFECT OF LIGHT ON PINEAL N-ACETYLTRANSFERASE DURING THE DARK PHASE OF THE CIRCADIAN CYCLE

Klein and Weller found that, when male Osborne-Mendel rats that were housed in a 14-hour dark:10-hour light room were removed during the dark phase and transferred to a room where the

lighting was about 100 lumen/m^2, the pineal NAT activity fell during a 10-minute exposure period from 5000 units to 400 units, with a halving time (t/2) of less than 3 minutes (88). They concluded that this quick change in NAT was not due to animal handling because animals that were blinded by bilateral enucleation before transferring to the light did not show such decrease in NAT as was seen in the experimental animals.

In their study using chick eyes and pineal glands, Binkley et al. found that when chicks were exposed to light for 1 hour during their subjective dark phase, NAT was rapidly suppressed in the pineal by 61-76% and in the eye by 36-59% (70). Hamm and Menaker found that bright light, given during the night when NAT levels are high, quickly inactivated NAT in chick retina; one hour of light (1400-1500 lux) given at midnight caused NAT activity to decrease to daytime levels, with a halving time of 10-12 minutes. In addition, this acute effect of light also caused chick retinal melatonin to decrease to daytime levels (72). Light during dark phase therefore appears to have an immediate effect on both pineal and retinal NAT activity.

In summary, it has been shown that melatonin is produced by both the pineal gland and the retina in certain mammals and, once released from the pineal and into the circulation, acts upon several peripheral organs to induce physiologic changes. Furthermore, melatonin and its biosynthetic enzymes appear to respond to changes in environmental illumination.

THE EFFECT OF LIGHT DURING DARK ON IOP

Unpublished data from Dr. Douglas S. Gregory at the Dept. of Ophthalmology, Yale School of Medicine, showed that, in normal rabbits previously entrained to a 12L:12D light cycle, when lights were turned on 1 hour after the onset of darkness, IOP dropped precipitously and, when lights were turned off, the IOP rebounded to the control dark value. This acute effect of light during dark on IOP was repeated one more time after returning to dark (Figure 8). The effect of light during dark on aqueous flow was not studied.

Since intraocular pressure and aqueous humor flow appear to respond to changes in environmental illumination as do melatonin and its biosynthetic enzymes, one might also speculate that there may be similar reactions of IOP and aqueous flow to the acute effect of light during dark as those of melatonin and its biosynthetic enzymes. This possible interaction forms the basis for our investigation.

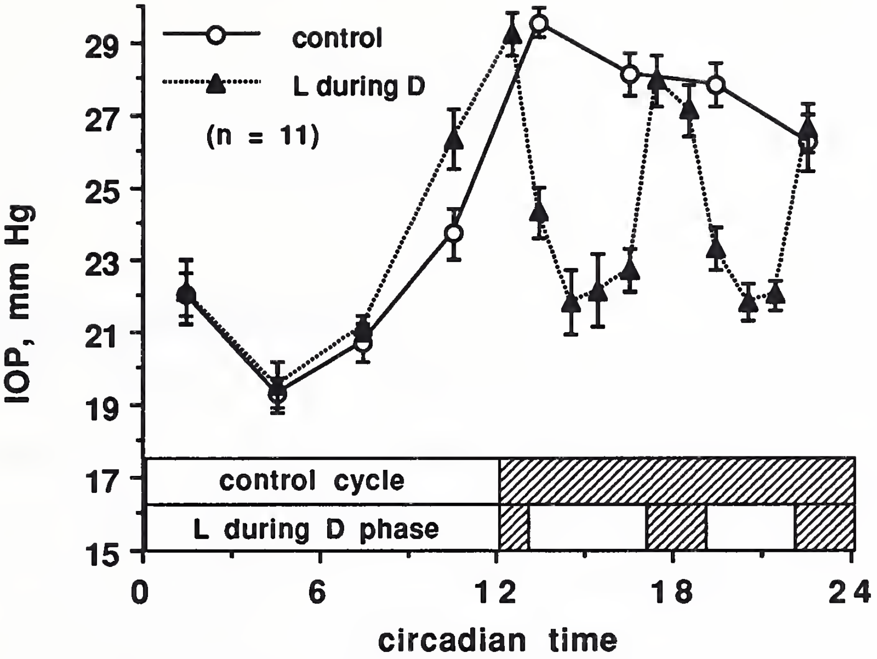

Fig. 8: The effect of light during dark on IOP in normal rabbits, n=11 (unpublished data from D. S. Gregory).

HYPOTHESIS

In summary, this study will attempt to answer the following 3 questions:

1. What is the acute effect on IOP of exposure of rabbits to light during the dark phase of the circadian cycle? (i.e. to repeat Gregory's experiment in a systematic way for purposes of obtaining paired data and reconfirming his results)
2. Does exposure to light during the dark phase of the circadian cycle decrease aqueous flow?
3. Is the sympathetic nervous supply to the eye required for the response of IOP to light during the dark phase of the circadian cycle?

MATERIALS AND METHODS

All procedures performed adhered to the ARVO Resolution on the Use of Animals in Research.

ANIMALS/ENTRAINMENT

New Zealand white rabbits weighing between 4-5 lbs each were housed in a light-tight room (with temperature maintained at 22 C and food and water provided ad lib) were entrained to a lighting cycle of 12L:12D for at least two weeks before experiments. Time is expressed as circadian time (CT) where 0:00 defines when the lights come on and 12:00 defines when lights go off.

SURGICAL PROCEDURES

Unilateral superior cervical ganglionectomy (uni-CGX), or prejunctional section of the cervical sympathetic trunk (decentralization, uni-DX), was performed on 7 or 8 rabbits, respectively, by Dr. Yoshiaki Kiuchi. Animals were anesthetized by intramuscular injection of 0.5 to 1 milliliters of 50 mg/mL xylazine and 50 mg/mL ketamine. After surgery, the animals were allowed to recover for approximately 2 weeks in 12L:12D. Cervical ganglionectomy was confirmed by application of 1% hydroxyamphetamine (89), which causes reversible release of norepinephrine and therefore pupil dilatation in the normal and DX eyes, but not the CGX eyes. Decentralization was confirmed by

application of 5% cocaine (89), which blocks re-uptake of norepinephrine from the nerve terminals and causes dilatation in the normal eyes, but not in the DX eyes (due to decreased release of norepinephrine from decentralized nerve terminals).

IOP Measurement

The rabbits used in this experiment included the normals, those which underwent uni-CGX, and those which underwent uni-DX. All animals used were entrained for at least 2 weeks in 12L:12D before the experiment. IOP measurements were performed with a previously calibrated DigiLab Micro-1 applanation pneumotonometer. When performed in the dark, the measurement was done with the help of the light from a Bright Lab Jr. red light. Two drops of an anesthetizing solution (1/10 dilution of Ophthetic in BSS) were applied before IOP measurement. The animal tolerated the procedure very well without signs of distress.

IOP measurements were made every hour from 12:30 CT to 19:30 CT on the first day. On the second day ("light during dark" experiment), IOP was measured at the same times, except that the light in the animal room was turned on at 13:00 CT, and remained turned on until 17:00 CT, when it was turned off. Therefore, IOP was measured at 4 time points (13:30 to 16:30 CT) in the light. Since the IOP from the two eyes are, on average, the same in both eyes, the IOP of each animal was recorded as the average of the two eyes. A paired student's t-test was used for statistical analysis.

FLOW MEASUREMENT

This experiment made use of the intravitreal depot method of flow measurement as devised by Johnson and Maurice (90), utilizing FITC-dextran as tracer. The validity of this method rests upon the following assumptions:

(1) that complete mixing of FITC-dextran in vitreous occurs;
(2) that a steady state exists, i.e., that the rate of diffusion of FITC-dextran from the vitreous into the aqueous is equal to the rate at which it is leaving the anterior chamber by bulk flow;
(3) that the rate of aqueous flow is constant;
(4) that the mass of FITC-dextran in the vitreous does not change appreciably during each measurement.

Under such conditions, flow can be calculated from the following equation:

$$\textbf{Flow} = k_{vit}\ (M_{vit})\ /\ C_{aq}$$

where:

k_{vit}=the coefficient of diffusion of FITC-dextran from the vitreous into the aqueous,
M_{vit}=the mass of FITC-dextran in the vitreous at the time of flow measurement, and
C_{aq}=the concentration of FITC-dextran in the aqueous at the time of flow measurement.

Relying on the work of Johnson and Maurice (90), Smith and Gregory (46) determined that, after injection, the FITC-dextran from the vitreous began diffusing into the aqueous and the concentration of fluorescein in the aqueous quickly reached a maximum within 2 days; thereafter, the fluorescein concentration of the aqueous decreased exponentially. The slope of (ln C_{aq}) versus time is equal to $-k_{vit}$. The value of k_{vit},, determined by Smith & Gregory, was

estimated by measuring FITC-dextran in the anterior chamber (C_{aq}) at the same time for 15 to 21 days beginning 5 or 6 days after intravitreal injection of FITC-dextran in both eyes of eight NZW rabbits entrained to 12L:12D. The value of k_{vit} was estimated to be 0.089 ± 0.005 day^{-1}. As will be discussed in a later section, however, the assumption of constant aqueous flow rate does not hold true in the rabbit because a circadian rhythm of flow exists.

Intravitreal Injection

Ten percent FITC-Dextran (MW 148,000, Sigma Chemical, St. Louis, MO), dissolved in distilled water, was sterilized by autoclaving for 30 minutes. Before injection (which was performed during the light phase for convenience), 1-2 drops of an anesthetizing Ophthetic solution were applied to the eyes, which were then proptosed. Twenty microliters of FITC-Dextran were injected, using a 30 gauge needle, into the vitreous at approximately 3 millimeters superolateral to the limbus, away from the cornea. No evidence of bleeding or inflammation were seen after each injection. The animals were then returned to their respective cages after injection, and entrainment was continued for another 7 days before flow measurement began. By then, the loss of the fluorescein from the aqueous should be first-order.

C_{aq} *Measurement*

Flow measurements were performed at 12:30, 14:30, and 16:30 circadian time (CT) on 2 successive days. A Coherent Fluorotron Master (Palo Alto, CA) standardized for FITC-Dextran was used to

measure the FITC-Dextran in the anterior chamber (C_{aq}). Measurement was first performed on the right eye, then the left. Flow measurement done on the first day served as controls. Flow measurements in the dark were facilitated by the use of a Bright Light, Jr., red light. Due to the fact that measurements for each time point were done in triplicate, however, actual time of measurement lasted from approximately 10 mintutes before to 10 minutes after each time point, and it was assumed that the fluorescein concentration in the aqueous was constant throughout a single time point of measurement, i.e. the first-order loss of fluorescein during this brief period was assumed to be negligible. On the second day the first flow measurement (12:30 CT) was performed in the dark, then, at 13:00 CT, the light in the animal room was turned on, and the next two measurements (14:30 & 16:30 CT) were done in the light.

Eye Enucleation and Vitreous Extraction

Approximately one hour after the last flow measurement of the second day (light during dark) of the experiment, the rabbits were killed by injection of an overdose (approximately 1 mL) of "Buthanasia-D" into the left marginal ear vein with a butterfly needle. The eyes were then enucleated with a curved-tip scissor and stored in -80 C. On the day of vitreous extraction, enucleated eyes of all animals from the flow experiment were removed from the -80 C freezer and warmed in a cold room at 4 C. Then, while the eye was still relatively frozen hard and easy to manipulate, sclera of each eye was shaved away with a scalpel and peeled off carefully. The frozen

vitreous, lens, and aqueous were then transfered into 5 mL of phosphate buffered saline extraction solution (0.85% NaCl, 0.075 M Na_2HPO_4, 0.025 M NaH_2PO_4) and left overnight on a rotating mixer in the 4 C cold room for 24 hours to extract FITC-dextran from the vitreous. The next day, tissue and extracted FITC-dextran were separated by centrifugation at 15,000 rpm for 10 minutes in a Sorvall SS34 rotor at 4 C. The volume of the resulting supernatant was measured (in mL), then the concentration (in ng/mL) of FITC-dextran was determined using the Coherent fluorophotometer. The mass of FITC-Dextran in the vitreous, M_{vit}, was then calculated by multiplying the volume of supernatant (in mL) by the concentration of FITC-dextran (in ng/mL) at the time of measurement to give a final value in nanograms (ng). Then M_{vit} was corrected for day 1 and day 2 of flow measurements by taking into account the exponential loss of fluorescein from the vitreous between the time of each flow measurement and the time of vitreous extraction, using the equation:

$$\mathbf{M_{vit,\ t=n} = \exp\ (k_{vit} \times N)\ \ X\ \ M_{vit,\ t=0}}$$

where

$M_{vit,\ t=n}$: mass of FITC-dextran in the vitreous at the time of flow measurement,
$M_{vit,\ t=0}$: mass of FITC-dextran in the vitreous at the time of vitreous extraction (equals M_{vit} from above),
N: the number of hours elapsed between the time of flow measurement and the time of vitreous extraction,
k_{vit}: the coefficient of diffusion of FITC-dextran from the vitreous into the aqueous (0.0037/hr, or 0.089/day).

For purposes of statistical tests, flow values from both eyes of each animal were averaged; therefore, sample size represented the number of individual rabbits rather than of individual eyes tested. A student's t-test was used for statistical analysis.

All of the procedures mentioned under the "material and method" section were performed by the author except the surgery (i.e. uni-CGX and uni-DX) performed by Dr. Yoshiaki Kiuchi. The graphs and tables presented in this thesis were the work of the author except figures 1-8 and 15.

RESULTS

EFFECT OF LIGHT DURING DARK ON IOP

Normal Animals

Eleven animals were used in this experiment. Exposing rabbits to light during the dark phase of the circadian cycle had the effect of rapidly decreasing IOP (Table 1) beginning 1/2 hour after light comes on (13:00 CT) by 5.5 mmHg at 13:30 CT (p<0.001); the average decrease from these 4 time points is 5.5 mmHg (see Figures 9 & 10). When lights were turned off at 17:00 CT, IOP rebound quickly to the control dark phase values at 17:30 CT, 18:30 CT, and 19:30 CT.

Table 1: The effect of light during dark on IOP (in mmHg) in normal rabbits (n=11).

Time (C.T.)	Day 1 (Dark control)	Day 2 (L during D)	L during D - D	SEM	p value
12:30	32.8	32	-0.8	1.1	
13:30	34.1	**28.5**	**-5.5**	0.9	<0.001
14:30	32	**26.5**	**-5.5**	1	<0.001
15:30	31.4	**25.6**	**-5.7**	1	<0.001
16:30	31.7	**26.6**	**-5.1**	1.2	<0.005
17:30	32.2	31.5	-0.7	1	
18:30	32.3	32.1	-0.2	0.9	
19:30	30.3	30.9	0.5	1.1	

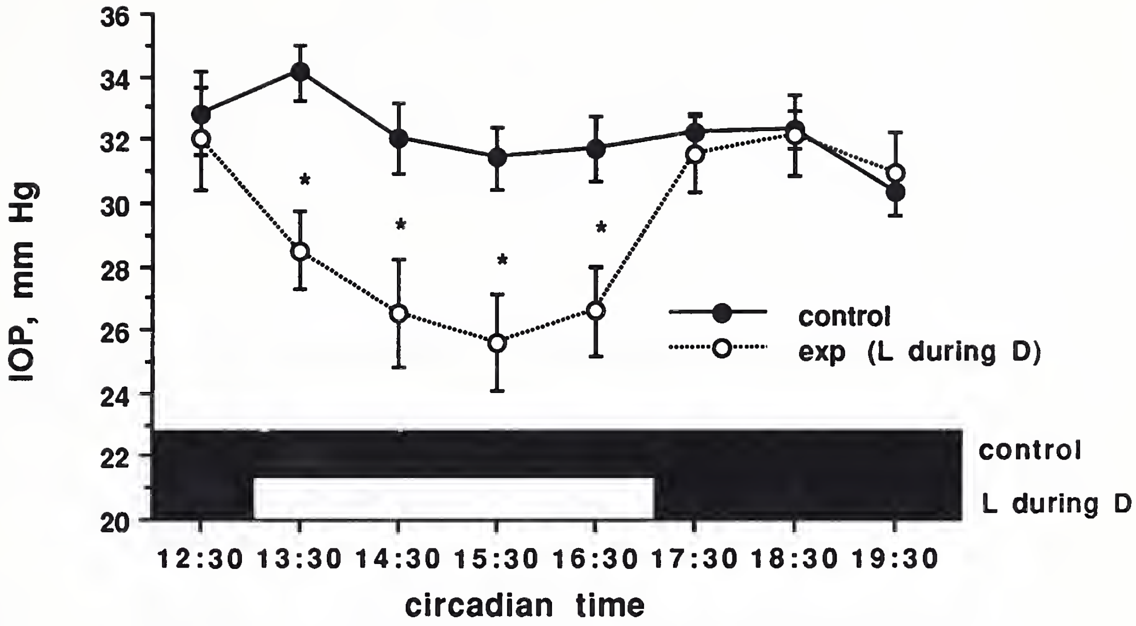

Fig. 9: The effect of light during dark on IOP; normals (n=11).

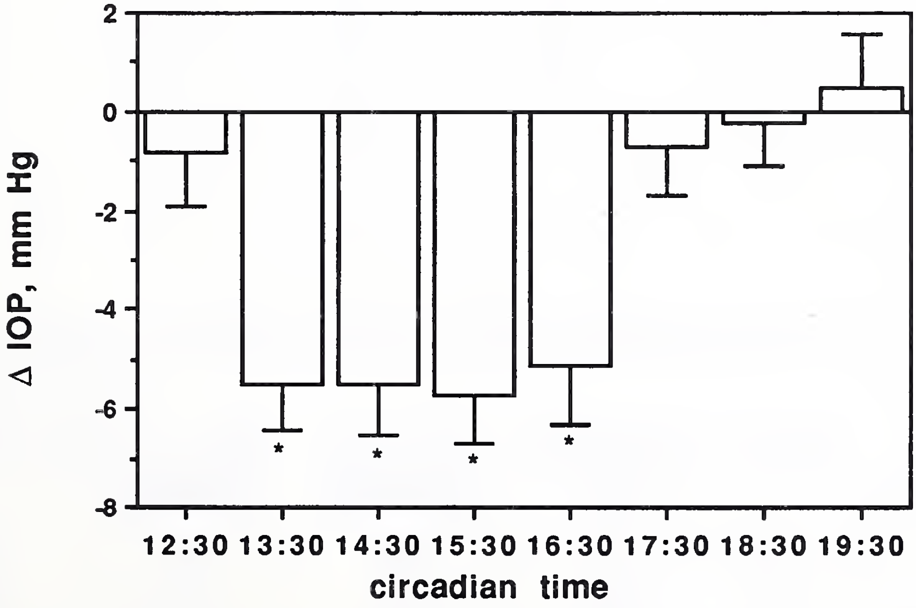

Fig. 10: The effect of light during dark on IOP in normals; IOP (L during D) - IOP (control D), n=11.

Unilaterally Decentralized Animals

In the 8 animals that had uni-DX, the control eye showed the same precipitous drop in IOP (Table 2) when light came on during

dark observed in control animals (Figure 9). In the DX eye, the effect on IOP was significant ($p<0.025$) at 14:30 CT only, with a 2.8 mmHg drop in pressure (Table 3 & Figure 11). Overall, the response of IOP to light during dark in the decentralized eye was suppressed.

Table 2: The effect of light during dark on IOP (in mmHg) in control eyes of uni-DX rabbits (n=8).

Time (C.T.)	Day 1 (Dark control)	Day 2 (L during D)	L during D - D	SEM	p value
12:30	29.6	31.3	1.71	0.5	<0.01
13:30	30.1	**26.8**	**-3.3**	1	<0.025
14:30	30.6	**25.1**	**-5.5**	0.8	<0.001
15:30	29.5	**24.7**	**-4.8**	1.5	<0.025
16:30	29.6	**25.1**	**-4.5**	0.5	<0.001
17:30	30.1	30.1	0.04	0.9	
18:30	29.7	30.3	0.6	0.5	
19:30	30	29.5	-0.5	0.8	

Table 3: The effect of light during dark on IOP (in mmHg) on DX eyes of uni-DX rabbits (n=8).

Time (C.T.)	Day 1 (Dark control)	Day 2 (L during D)	L during D - D	SEM	p value
12:30	22.1	23.6	1.5	0.7	
13:30	23.1	**23.1**	**0.01**	0.9	
14:30	24.7	**22**	**-2.8**	0.8	<0.025
15:30	23.7	**22.6**	**-1.1**	1.2	
16:30	22.7	**22.3**	**-0.4**	1.5	
17:30	22.1	22.1	-0.03	1.3	
18:30	21.9	22.7	0.9	1.1	
19:30	21.8	20.6	-1.3	0.6	

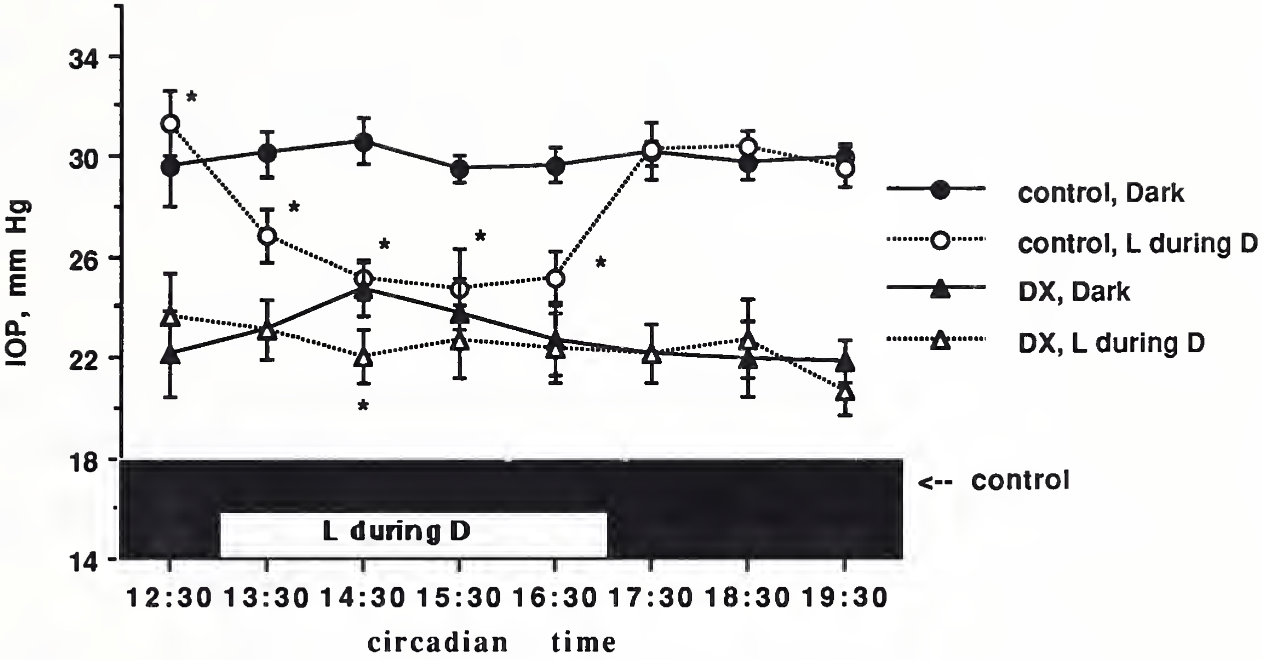

Fig.11: The effect of light during dark on IOP in unilaterally decentralized rabbits (n=8).

Note that in the control eye, there was a statistically significant difference in the IOP between first time points (12:30 CT) of the first and second day when no difference was expected. However, since the second-day (L during D) value is 1.7 mmHg higher than on first day, one can see that this would only further exaggerate the effect of light during dark on IOP if one were to normalize the IOP values at 12:30 CT.

Unilaterally Ganglionectomized Animals

In the seven animals which had uni-CGX, the control eye again exhibited the rapid drop in IOP when exposed to light during the dark (Table 4). In the CGX eye, however, no significant IOP changes

were observed at any time points except at 12:30 CT in the dark (Table 5), when no difference was expected (Figure 12). Overall, superior cervical ganglionectomy suppressed the IOP response to light during dark.

Table 4: The effect of light during dark on IOP (in mmHg) in control eyes of uni-CGX rabbits (n=7).

Time (C.T.)	Day 1 (Dark control)	Day 2 (L during D)	L during D - D	SEM	p value
12:30	29.8	32.8	3	0.5	<0.005
13:30	30.5	**27.6**	**-2.9**	0.8	<0.025
14:30	30.8	**22.6**	**-8.1**	1.8	<0.005
15:30	29.6	**21.9**	**-7.7**	1.9	<0.01
16:30	29.6	**23.8**	**-5.9**	2.5	
17:30	29.8	29.6	-0.1	1	
18:30	28.2	30.5	2.3	2.1	
19:30	27.3	30.8	3.4	0.7	<0.005

Table 5: The effect of light during dark on IOP (in mmHg) in CGX eyes of uni-CGX rabbits (n=7).

Time (C.T.)	Day 1 (Dark control)	Day 2 (L during D)	L during D - D	SEM	p value
12:30	21.8	23.5	1.7	0.4	<0.005
13:30	22.4	**25.1**	**2.7**	1.8	
14:30	21.1	**20.3**	**-0.7**	1.8	
15:30	21.6	**19.6**	**-2**	1.4	
16:30	20.2	**18.9**	**-1.3**	0.9	
17:30	21.8	20	-1.9	1.3	
18:30	21.8	20.7	-1.2	1.3	
19:30	20.9	21.5	0.6	0.8	

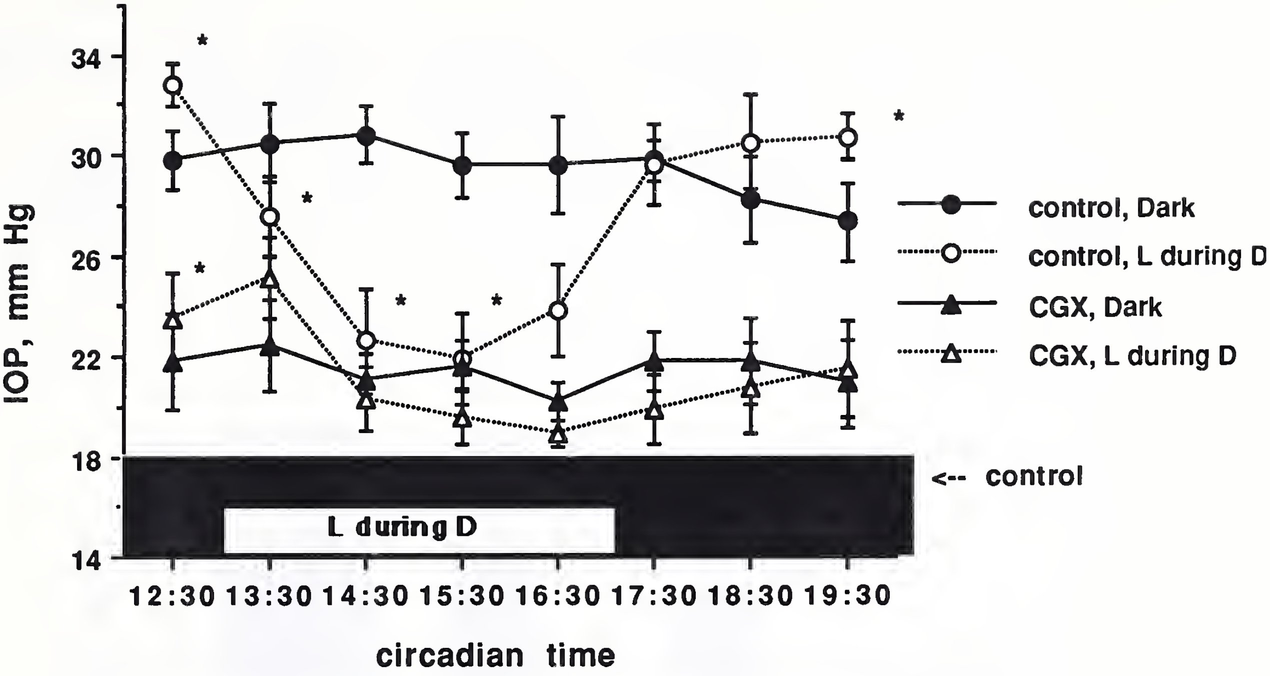

Fig. 12: The effect of light during dark on IOP in unilaterally ganglionectomized animals (n=7).

EFFECT OF LIGHT DURING DARK ON AQUEOUS FLOW

Of the control eyes injected with FITC-dextran in this experiment, 4 eyes belonging to 3 animals had k_{vit} values less than 1/5th the mean k_{vit} of all 24 eyes (before averaging the 2 eyes of each animal), indicating that in these four eyes the loss of fluorescein from anterior chamber had not reached an exponential decay on the first day of flow measurement. The results from these 3 animals were therefore deleted from the final calculation. Flow rates (in ul/min.) were calculated at three different time points (12:30, 14:30, and 16:30 CT) in each of the two days of the experiment (Table 6 & Figure 13).

Table 6: The effect of light during dark on aqueous flow (in ul/min.) in normal rabbits (n=11).

Time (C.T.)	Day 1 (Dark control)	Day 2 (L during D)	L during D - D	SEM	p value
12:30	2.3	2.5	0.2	0.08	<0.025
14:30	2.4	2.4	0.02	0.06	
16:30	2.1	2.2	0.07	0.09	

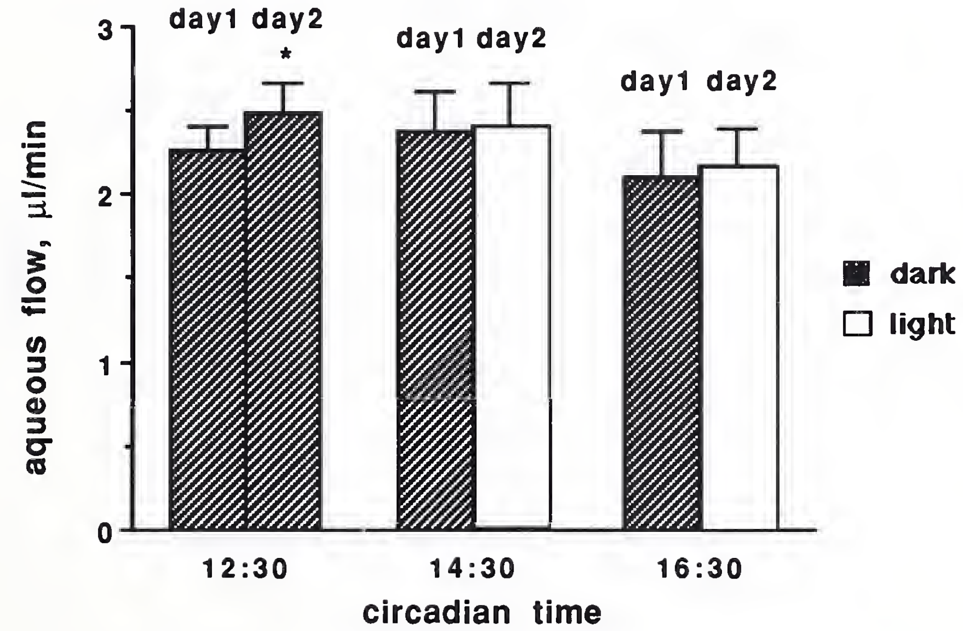

Fig. 13: The effect of light during dark on aqueous flow in normal animals using intravitreal depot method (n=11).

As can be seen in Figure 13, there was a statistically significant ($p<0.025$) difference between the first time points (12:30 CT) of day 1 (dark control) and day 2 (light during dark) when no difference was expected, since both time points represented flow in the dark. To correct for this difference, aqueous flow values were normalized to this first time point (12:30 CT), and the results are presented in Table 7 & Figure 14.

Table 7: The effect of light during dark on aqueous flow (in ul/min.) in normal rabbits (n=11); normalized to 12:30 CT.

Time (C.T.)	Day 1 (Dark control)	Day 2 (L during D)	L during D - D	SEM	p value
12:30	1	1	0	0.03	
14:30	1.1	**0.96**	-0.09	0.02	<0.005
16:30	0.9	**0.87**	-0.06	0.04	

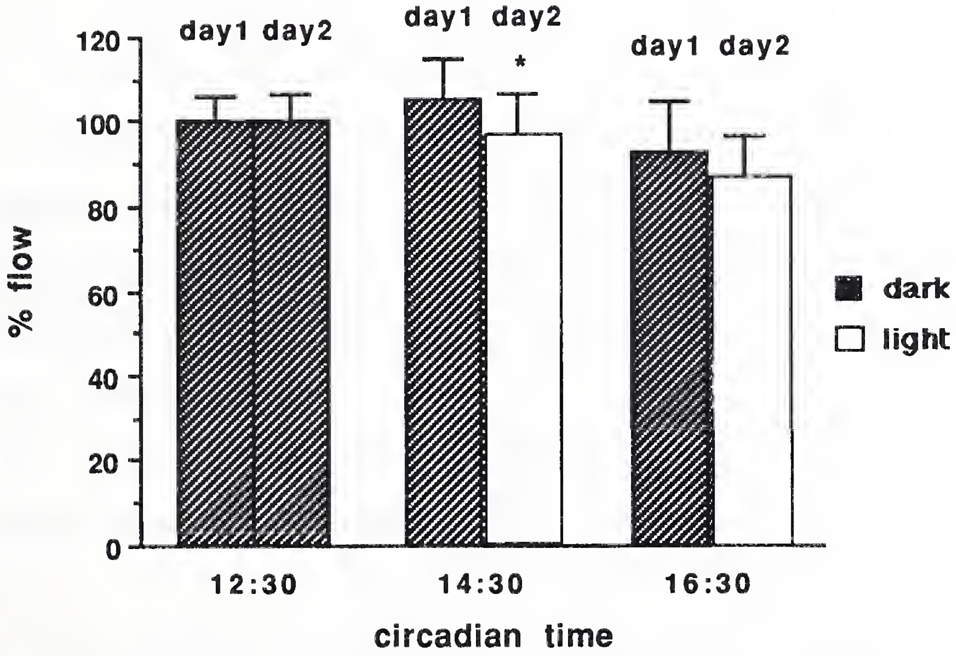

Fig. 14: Aqueous flow normalized to flow at 12:30 C.T.

After normalization, there was a small but statistically significant difference (p<0.005) between the flow on the second time point (14:30 CT) of first and second day, but the third time point (16:30 CT) was not statistically significant. Overall, aqueous flow as measured by the intravitreal depot method did not show a significant change when rabbits were exposed to light during the dark phase of the circadian cycle.

DISCUSSION

The results from these experiments indicate that, during the dark phase of the circadian cycle, exposure to light causes IOP to decrease rapidly and reversibly. They further show that this response to light is greatly suppressed in rabbits who previously underwent either unilateral superior cervical ganglionectomy or unilateral "decentralization," indicating that an intact sympathetic supply is necessary for this response to occur. In addition, whereas it has been shown that a circadian rhythm of aqueous flow underlies the circadian rhythm of IOP in rabbits (46), the response of IOP to light during the dark is not accompanied by a similar response of flow; therefore, the decrease in IOP appears not to be mediated by decreased aqueous flow.

One explanation for these observations is that the decrease of IOP results from light-mediated constriction of the pupil; pupillary constriction is known to increase the facility of outflow of aqueous through the trabecular meshwork, as pilocarpine--a parasympathetic agonist which causes pupil constriction--is used as an antiglaucoma medication. In their study on circadian rhythm of IOP in NZW rabbits, Katz et al. (25) noted "on several occasions that the IOP would rapidly decrease when pupillary constriction occurred. One rabbit showed a decrease in IOP from 16 to 12 mm Hg. This decrease occurred within 60 seconds as the pupil constricted." The observed drop in IOP in their experiment possibly reflects what is observed in the present experiment, since increased outflow facility without a change in production of aqueous--while causing a drop in

IOP--will result in no change in the fluorescein concentration in the anterior chamber. In the uni-DX and uni-CGX eyes, the sympathetic innervation to the eye was interrupted, resulting in pupil constriction, which leads to increased outflow facility and decreased IOP chronically, therefore making IOP relatively unresponsive to light; in the contralateral normal eye, however, a drop in IOP was observed when the normal pupil constricts, indicating that the mechanism of decreased IOP must be facilitated locally, not systemically. To further elucidate the underlying mechanism, one can perform an experiment where normal rabbits are given topical tropicamide eye drops (a mydriatic agent) during the dark phase before being subjected to light and observe whether IOP would still decrease after light comes on. If pupil constriction does play a role in the acute drop of IOP as we suspected, then this experiment would show no acute IOP drop, since the pupils were made to dilate (by tropicamide) and would not respond (or constrict) to light during dark.

In a detailed analysis of various methods employed by different researchers to measure aqueous flow in rabbits, Smith (91) showed that one assumption made by the intravitreal depot method devised by Johnson and Maurice (90)--that the rate of aqueous flow is constant--is erroneous due to the fact that a circadian rhythm of flow exists in this specie. This error is particularly evident if aqueous measurements are made shortly after rapid changes in flow rate. The error also seems to be magnified in the intravitreal depot method because, in a study comparing the corneal depot and the intravitreal depot methods of flow measurement, Gaul and Brubaker

(92) found that the corneal depot method was able to detect significant changes in aqueous flow in rabbits after separate administrations of IV acetazolamide, IV mannitol, and water loading that were not detectable by the intravitreal depot method, and concluded that "while the vitreous depot method is probably superior for measuring sustained changes of the rate of aqueous flow over tens of hours or days, ... it cannot be used for measuring changes over shorter periods." Using a compartmental model of a circadian system, Smith concluded that "the presence of a continuously changing rate of aqueous flow results in underestimation of the range of the circadian rhythm of aqueous flow and a shift in the phase of the rhythm. This result occurs because the changing concentration of FITC-dextran lags significantly behind the changing rate of aqueous flow, due to the low rate of turnover of aqueous of about 1 percent per minute." Even if this is true, a turnover rate of 1% per minute would result--in 100 minutes-a 100% turnover, a change which was still not detected in our experiment that lasted 3 hour and 30 minutes from the time the light was turned on (13:00) until the last flow measurement (16:30). Therefore, it is unlikely that the intravitreal depot method that we used in this experiment is at fault for not able to detect changes in aqueous flow. Furthermore, Kiuchi et al. (35) and Yoshitomi et al. (47) using the same intravitreal depot method, were able to show apparent changes in flow over a similar time period after timolol or bunazosin treatment.

Kiuchi and Gregory had subsequently repeated our experiment in normal animals to determine whether the corneal depot method

can detect earlier changes in flow in our experiment that was not detected by the intravitreal depot method (as Gaul and Brubaker's study suggested). The results, indicated in Figure 15, shows a paradoxical increase in flow which is not statistically significant; therefore, the possiblility of a change in flow in the dark as effected by light is not detected by either the corneal or the intravitreal depot methods of flow measurement.

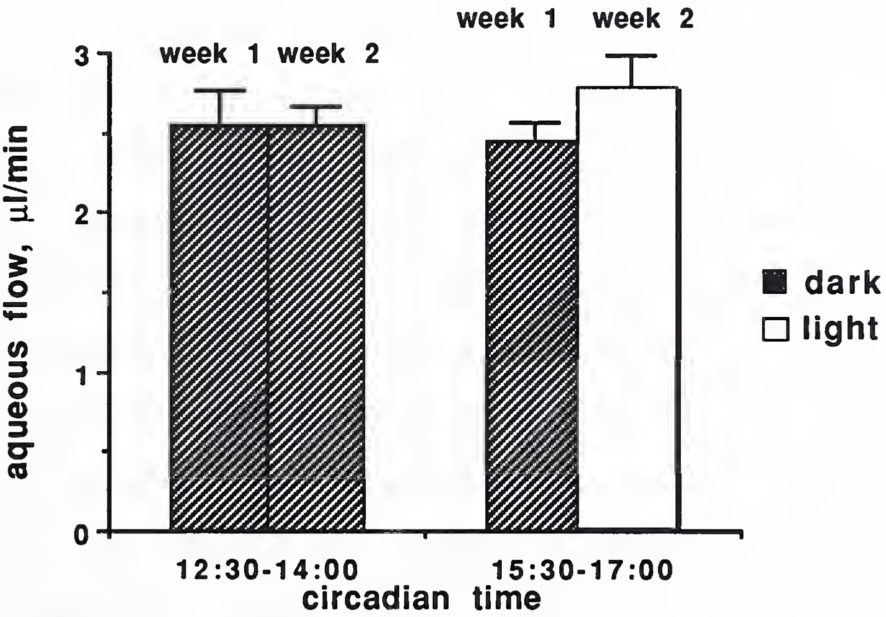

Fig. 15: The effect of light during dark phase on aqueous flow in normal animals (corneal depot method), n=8.

Uveoscleral outflow, defined as aqueous outflow through the intermuscular spaces of the ciliary muscle, into the supraciliary-suprachoroidal space, and out through the substance of the sclera or through the perivascular spaces of the emissarial channels, represents another exit pathway for aqueous humor (93). Uveoscleral outflow is largely pressure-independent and has been

demonstrated in monkeys (94, 95), humans (96), rabbits (97), cats (98), and dogs (99). If uveoscleral outflow were to contribute to any change in aqueous flow in this experiment, i.e. if an increase in outflow through the trabecular meshwork (which is pressure-dependent) were accompanied by a decrease in uveoscleral outflow such that overall the flow did not change, then it is possible that one sees no change in flow as measured by our method. In rabbits, uveoscleral outflow at normal IOP was determined to be 1.1 ul/min. (97), which corresponds to 10% of total outflow; this value--or any fluctuation around this value--may be significant as far as being able to mask or counteract any significant change in outflow through the trabecular meshwork. Therefore, decreased uveoscleral outflow may be a contributor to the observed flow in our experiment.

In summary, various physiologic process have been shown to exhibit rhythmic changes synchronized to different environmental cues. A circadian rhythm of pineal and retinal melatonin and its precursor and biosynthetic enzymes--serotonin, Hydroxy-indole-O-methyl-transferase, and N-acetyltransferase, respectively--in the vertebrates have been linked to variation in environmental lighting, while the same is true of that of intraocular pressure and aqueous flow. Given that previous studies have shown rapid effect of light during the dark phase of the circadian cycle on the concentrations of the above indoleamines and enzymes, and the effect of melatonin on IOP, this experiment has demonstrated an analogous effect of light during dark on IOP in rabbits, while its effect on aqueous flow remains insignificant. The underlying mechanism responsible for the drop in IOP in this experiment remains to be elucidated. Because

light has an effect of decreasing IOP during the dark phase, one might explain the reason why Katz et al. (25) (see "Circadian Rhythm of IOP" section) did not see a rise in IOP during the dark is that, during measurement, the light remained turned on for an additional 6 hours (lights off @ 23:30) beyond that which the animals were previously entrained to (lights off @ 17:30). In addition, our finding may also explain why Vareilles et al. (26) did not see a greater range of IOP in rabbits housed in constant light.

In conclusion, light has 2 effects on IOP in rabbits: (1) it sets the phase of the circadian rhythm of IOP, (2) it rapidly and reversibly decreases IOP during the dark phase of the circadian cycle. It then follows that it is important to avoid even brief exposure to light during the dark phase of the circadian cycle when using the rabbit model for IOP studies. Furthermore, the results may explain the failure of some earlier investigators to observe a circadian rhythm of IOP in rabbits.

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